BMJ Open

Probiotics influencing response of antibodies over time in seniors after COVID-19 vaccine (PIRATES-COV): a randomised controlled trial protocol

Jean-Charles Pasquier,[1,2] Mélanie Plourde,[3] Sheela Ramanathan,[3] N Chaillet,[4] Guy Boivin,[4] Isabelle Laforest-Lapointe,[3] Hugues Allard-Chamard,[3] Geneviève Baron,[3] Jean-François Beaulieu,[3] Tamas Fülöp,[3] Mélissa Généreux,[5] Benoît Mâsse,[6] Julie Robitaille,[4] Louis Valiquette,[7] Sarah Bilodeau ,[2] Danielle H Buch,[8] Alain Piche[3]

To cite: Pasquier J-C, Plourde M, Ramanathan S, *et al*. Probiotics influencing response of antibodies over time in seniors after COVID-19 vaccine (PIRATES-COV): a randomised controlled trial protocol. *BMJ Open* 2025;**15**:e088231. doi:10.1136/bmjopen-2024-088231

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (https://doi.org/10.1136/bmjopen-2024-088231).

Received 30 April 2024
Accepted 27 February 2025

For numbered affiliations see end of article.

Correspondence to
Dr Jean-Charles Pasquier; jean-charles.pasquier@umontreal.ca

ABSTRACT

Introduction The elderly are particularly vulnerable to morbidity and mortality from COVID-19, the disease caused by the SARS-CoV-2. Approximately 20% of the elderly showed no antibodies 3–5 months post-second dose of the COVID-19 vaccine. As probiotics have been shown to increase influenza-specific antibody levels post-influenza vaccination, we aim to reduce the percentage of participants without antibodies against the SARS-CoV-2 spike protein receptor-binding domain (anti-S1-RBD) at 6 months post-vaccination.

Methods and analysis Our study design is a double-blind randomised controlled trial, using intention-to-treat analysis. Eligible participants are a purposive sample of 688 adults aged 65–89 years, in Quebec, Canada, not diagnosed with COVID-19 in the 3 months prior to recruitment and who wish to receive a government-recommended mRNA booster (Pfizer-BioNTech, Moderna) vaccine. The intervention consists of one capsule/day of a probiotic dietary supplement of *Lacticaseibacillus rhamnosus* and *Lacticaseibacillus casei* 6×10^9 CFU/capsule or a placebo, for 15 days pre-booster and post-booster vaccine. All participants provide dried blood spot samples at three timepoints (inclusion, 3 and 6 months post-vaccination) and a stool sample for microbiome analysis. A subgroup of 100 participants living near Sherbrooke, Quebec, is expected to volunteer for two onsite blood-test visits (at inclusion and 6 months post-vaccination). The primary outcome is the percentage of participants without anti-S1-RBD antibodies at 6 months post-vaccination. Secondary outcomes include longitudinal analysis of anti-S1-RBD and anti-N antibodies at three timepoints. In the subgroup, serum levels of neutralising antibodies will be determined at inclusion and 6 months post-vaccination. Probiotic and vaccine side effects are monitored. At the end of the study, we expect to identify the adjuvant effect of probiotic on vaccine-induced immune response.

Ethics and dissemination The study was approved by Research Ethics Board of the *Centre Intégré Universitaire de Santé et des Services Sociaux de l'Estrie- Centre Hospitalier Universitaire de Sherbrooke* (*CIUSSS de l'Estrie-CHUS*) and the *CHU de Québec-Université Laval # MP-31-2022*-4598 as well as Health Canada. All participants will provide informed consent. Results will be disseminated to the scientific community and to all networks related in this research.

Trial registration number NCT05195151.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is the first randomised controlled trial to evaluate the interaction between oral probiotics and the durability of COVID-19 vaccine protection in the elderly.
⇒ Probiotics are already available at low cost without prescription in Canada and other countries, suggesting a cost-effective and easily implementable option worldwide.
⇒ Recruiting to target sample size will be a challenge mainly because participants have to be contacted 3–4 weeks before their booster dose appointment (for 2 weeks of probiotic/placebo intake before vaccination).
⇒ Maintaining adherence during a 6-month study with multiple sample collection is a challenge as well.

INTRODUCTION

The elderly population is particularly at risk of morbidity and mortality from COVID-19, the disease caused by the SARS-CoV-2.[1–3] Vaccination against COVID-19 began in Canada in 2021, mainly using the mRNA vaccines by Pfizer-BioNTech and Moderna. However, due to waning immunity and subsequent large-scale infection by SARS-CoV-2 delta and omicron variants,[4] booster doses have proven necessary.[5] While two doses of vaccine induced robust activation of cell-mediated and humoral immune response,[6 7] the magnitude was less than that conferred by natural COVID-19 infection.[8–10] A recent

meta-analysis showed that COVID-19 vaccines in the elderly were effective and tolerated, with a decrease in COVID-19-related deaths.[11] Nonetheless, 3 months after vaccination, antibody levels declined,[12] at a faster rate in the elderly than in the general population.[1 2] Three to five months after the second dose, 20% of older adults showed no trace of anti-COVID antibodies.[1 13 14] Many countries, including Canada, have offered booster doses every 5 or 6 months. A high frequency of booster administration is a strain on public health resources and increases societal and economic costs. COVID vaccine durability is thus a public health concern.

The human microbiota, defined as all microorganisms colonising the human body in the gut and oral cavity, are known to play a role in host protection, from tight junction regulation in the intestinal mucosa to the production of short-chain fatty acids to increasing innate and acquired immunity.[15–18] Microbiota involvement has been reported in digestive, respiratory, autoimmune and metabolic diseases.[16] In the elderly, gut microbiota showed less diversity, lower levels of beneficial bacteria and higher levels of pathogenic species.[19] Furthermore, the use of antibiotics reduced immune response to vaccine boosters in individuals with low titres of existing antibodies.[20] If gut microbial dysbiosis (ie, a state of imbalance leading to a loss of diversity or a change in community composition) was to partially explain differential response to vaccination, intervention with probiotics might improve vaccination effectiveness.[21]

Two meta-analyses reported improved response to the influenza vaccine with probiotic coadministration,[22 23] as has a randomised controlled trial in the elderly.[24] Nevertheless, it remains difficult to draw clear conclusions on the benefits of probiotic coadministration with vaccination based on these studies, as they included different sample sizes, different strains and doses of probiotics, and different modes of administration and lengths of treatment.[25] A recent trial on probiotics and COVID-19 vaccination in nursing home residents showed conflicting results, with a trend towards positive effects.[26] Three trials on probiotic coadministration with COVID-19 vaccination are currently registered on clinicaltrials.gov.[27] We hypothesised that probiotics taken at the time of the COVID-19 booster dose would improve both humoral (antibodies to the SARS-CoV-2 receptor-binding domain) and cell-mediated (T and B cells) immune response in the elderly. This would increase immunity in the medium term and allow for longer spacing between booster doses. Further, increased memory cell immunity might prove protective against future variants.

To test this hypothesis, we will conduct a randomised controlled trial using a two-strain probiotic dietary supplement as adjuvant to the COVID-19 vaccine booster dose, with the aims of reducing the percentage of elderly without antibodies to the SARS-CoV-2 spike protein at 6 months post-vaccination. Our secondary objectives include a longitudinal analysis of antibodies at inclusion and at 3 and 6 months post-vaccination (dried blood spots), as well as a comparison of antibodies and spike-specific T and B cell levels at inclusion and at 6 months post-vaccination in a subset of participants. Further, we will also compare the proportion of confirmed cases of COVID-19 (by rapid antigen testing or PCR with symptoms and the proportion of COVID-19 asymptomatic cases in each study group). We will also monitor local and systemic side effects.

METHODS

Study design and population

The study design is a randomised controlled trial, using intention-to-treat analysis. The intervention consists of a probiotic supplement, as compared with an inert placebo, for enhancing immune response in the elderly for a period of up to 6 months following COVID-19 vaccination. The trial is to be conducted throughout Quebec (Canada) and coordinated by our study site at the *Centre de recherche du Centre hospitalier universitaire de Sherbrooke* (CR-CHUS). A convenience sample of 100 participants living in the Eastern Townships (Estrie), Quebec, in addition to taking part in the proposed study, will provide onsite blood samples for additional serological testing as part of the proposed study, that is, if participants are able to travel to the CR-CHUS, they will be invited for two in-person blood tests, until 100 subgroup participants are reached. With the COVID-19 situation in constant flux, the targeted booster vaccination is defined as the next booster dose of the mRNA COVID-19 vaccine (Moderna or Pfizer), as recommended by Quebec Public Health.

Inclusion/exclusion criteria

Adults aged 65 to 89 years, of either sex, living at home or in independent-living retirement homes in Quebec, Canada, are eligible for participation if they wish to receive the next government-recommended COVID-19 mRNA vaccine booster dose, if they have already received three vaccine doses (Pfizer-BioNTech or Moderna) and if the latest dose was at least 5 months prior to inclusion. Other inclusion criteria are telephone and/or internet access and cognitive ability to provide informed consent. The latter will be assessed using the Functional Activities Questionnaire;[28] participants with scores ≥9 will be excluded. Other exclusion criteria are allergy (soy, lactose, yeast, maltodextrin), chronic immunodeficiency/immunosuppression (eg, concurrent cancer chemotherapy or radiotherapy; concurrent active treatment for intestinal disorders such as duodenal ulcers, coeliac disease, ulcerative colitis, and Crohn's) and use of probiotics and/or antibiotics at the date of inclusion. Individuals with a serious condition precluding safe participation to the end of the study are excluded, as well as those having had COVID-19 in the past 3 months (confirmed clinically or, in the case of the 100-people subgroup, a posteriori by serological testing). Individuals who speak neither French nor English are excluded.

Recruitment, screening and consent

The study will be advertised at community centres and on social media, with a telephone number for callback, as well as through word of mouth from participants already registered. Different recruitment strategies will be used, including advertising in traditional media, information meetings in retirement homes and contacting potential participants from professional community lists.

Preliminary eligibility will be determined online or by telephone. A research assistant will then call potential participants, to explain the study, answer questions and blind telephone recordings.

Randomisation

Participants are randomised 1:1 into the placebo or intervention group, using permuted block randomisation stratified by age (65–79 years, 80–89 years) and sex (male, female). Randomisation will be programmed by the Applied Clinical Research Unit (URCA) of the *Centre de recherche Azrieli of the Centre Hospitalier Universitaire de Sainte-Justine* and performed online using REDCap.

The study will be double-blinded. Until unlocking of the database at study end, neither the participants nor the research team, partner or affiliated laboratories will be informed of the participants' group allocation. Pharmacists at CR-CHUS will prepare study products (probiotics or placebo) as per REDCap blinding requirements, using investigational products provided by Lallemand Health Solutions Inc. (Mirabel, Quebec, Canada) prior to study start.

Intervention

The intervention consists of an oral probiotic dietary supplement containing two bacterial strains of *Lacticaseibacillus rhamnosus* and *Lacticaseibacillus casei* 6×10^9 CFU/capsule or a placebo. Capsules for both probiotic and placebo are otherwise identical.

All participants take one capsule of the investigational product (probiotic or placebo) per day for 30 days: 15 days before and 15 days after receiving the COVID-19 vaccine booster shot. Should the vaccination appointment be delayed by more than 3 days once treatment has begun, treatment will continue until 15 days post-vaccination. An extra bottle of 35 capsules is kept for each participant at the hospital pharmacy, if needed.

Outcome measures

Primary outcome measure

The primary outcome measure is the percentage of participants without detectable antibodies against the SARS-CoV-2 spike protein receptor-binding domain (anti-S1-RBD) at 6 months following the vaccination booster dose. Anti-S1-RBD antibodies prevent binding of SARS-CoV-2 to the ACE-2 receptor and thus correlate well with neutralising antibodies (NAb) and vaccine effectiveness.[29] Levels of anti-S1-RBD antibodies will be determined by colorimetric ELISA on dried blood spot samples. As per the manufacturer's guidelines, the positivity threshold is set at 3 SD above the mean of negative controls (optical density on colorimetric blotting paper). ELISA tests have 95% sensitivity and 100% specificity.[29]

Secondary outcome measures

Secondary outcome measures are a longitudinal analysis of anti-S1-RBD and anti-nucleocapsid (Anti-N) antibodies at three different timepoints (inclusion; 3 and 6 months post-vaccination). Possibly, a quantitative variable will also be available. The analysis will be performed as described above on dried blood spot samples.

We will also compare the percentage of confirmed cases of COVID-19 with and without symptoms in each group (COVID-19 confirmed by rapid antigen testing or PCR). The analysis will be done using the reported adverse events and presence of antibodies Anti-N.

Another secondary outcome is the level of NAb and spike-specific T and B cells, at inclusion and at 6 months post-vaccination, in the subset of 100 participants providing venous blood samples. Lymphocyte binding to the spike protein will be measured by flow cytometry as described.[30 31] Absence of COVID-19 infection within the previous 3 months will be assessed by ELISA against the viral nucleocapsid.[32 33]

Subsequent analyses will include the comparison of gut microbial communities in both study groups at baseline, using stool samples provided by all participants at the time of inclusion. DNA will be extracted from stool samples and then sequenced using shotgun metagenomic sequencing on a NovaSeq 6000 platform (Illumina, Genome Quebec, Montreal, Canada). The bioBakery workflow (Huttenhower Lab, Massachusetts, USA) will be used for computer analysis. Shotgun sequencing data will go through metagenomic phylogenetic analysis (MetaPhlAn V.3.0) to obtain a profile of microbial communities (bacterial, archaeal, viral, eukaryotic).

Clinically, we will monitor side effects of the investigational product as well as those of the vaccine, both local (pain or redness at the injection site, swollen lymph nodes) and systemic (fever, fatigue, headache, muscle aches, digestive troubles, flu-like symptoms). We will also take note of COVID-19 infections (FLU-PRO questionnaire,[34] followed by COVID-19 rapid testing).

Changes in outcome measures

Secondary outcomes have been modified to add an Anti-N antibody analysis. This type of analysis was not available when the protocol was first written and seems to be essential to giving more answers about the proportion of COVID-19 infections.

Contamination bias

To prevent potential contamination bias, as probiotics are widely available and potential benefits assumed by the general population, participants are asked not to use probiotics (other than the investigational product) for the duration of the study. Questionnaires on probiotic use are completed before and during the study. Participants

found to have used over-the-counter probiotics will be documented but retained in study analyses.

Data and biological samples—collection and storage

For most participants, there are no in-person study visits. Biological samples are collected at home. These include one baseline stool sample and three dried blood spot samples: at inclusion, at 3 months post-vaccination and at 6 months post-vaccination. Blood spot samples are done by finger-prick testing on blotter paper; stool samples are self-performed. All samples are done at home and sent in by courier. Should any participants require help with sampling, a research team member will be available by phone or in person, upon request.

For a subset of 100 participants in the Sherbrooke area, there will be two in-person visits, one at baseline prior to starting any treatment and one at 6 months after vaccination. Venous blood collection will be done by a nurse at both visits, using the same brand of vacuum blood collection tubes with EDTA for anticoagulation. For each participant, five 4 mL tubes of blood will be collected. The final visit will include a satisfaction questionnaire regarding the research team, recruitment process, and acceptability of data and specimen collection. To decrease loss to follow-up, for those with mobility issues or if requested by participants, nurses will collect dried blood spot samples at the participant's home.

Biological samples

Dried blood spot samples will be couriered to the Sherbrooke study site, then sent to Guy Boivin's lab in Quebec City for analysis. Venous blood samples will be processed and stored at the Sherbrooke study site (S. Ramanathan). Stool samples will be couriered and stored in A. Piché's lab, sent to Genome Quebec for analysis, and interpreted at I. Laforest-Lapointe's lab in Sherbrooke.

Data collection

At the time of enrolment, questionnaires will be completed. These include questions on sociodemographic data, height and weight, ethnicity, vaccination status, current medications, medical history and comorbidities such as cancer. A validated food frequency questionnaire[35] will assess dietary habits.

Information on investigational product intake will be collected every 2 weeks for the first 33 days, online or by telephone. This will help in ensuring adherence to treatment and will be used to monitor adverse effects, if any.

Information on booster vaccination will be collected once a week, for 2 weeks. This includes local and systemic side effects, such as injection site pain/redness, fever, muscle aches, digestive troubles and flu-like symptoms.[36]

Any changes to health will be collected once a month. Participants infected with COVID-19 will remain in the study and fill out questionnaires describing symptoms. In the case of hospitalisation or death, data will be extracted from the participant's Quebec Health Record.

Data confidentiality and databank/biobank security

REDCap questionnaires are encrypted and kept at the URCA of the *Centre de recherche Azrieli of the Centre Hospitalier Universitaire de Sainte-Justine*. Data will be coded and the code kept by the principal investigator. Biological samples are collected in accordance with international standards.[37 38] Samples will be stored at the *Banque québécoise de la COVID-19* (BQC19) lab,[39] under the responsibility of the co-investigator.

Adverse events and data monitoring

Other than bloating, intestinal irritation or stool softer than usual, very few side effects are associated with probiotic use, although caution is warranted in critically ill patients.[40] Vaccine safety and tolerance are well established in the elderly. A four-member monitoring committee chaired by Bruno Piedboeuf will be advised of all adverse events (serious adverse events, within 48 hours; others, every 3 months). The steering committee will meet every 2 weeks. A scientific committee will meet monthly for 4 months and bimonthly thereafter. No interim analysis is planned.

COVID-19 infections

In the event of COVID-19 infection during the trial and prior to administration of the booster shot (as determined by rapid COVID tests upon declaration of symptoms), participants will be asked whether they wish to delay the booster for several months or forego it altogether. In either case, participants will remain in the trial. Investigational product regimen and data/sample collection will begin anew 2 weeks prior to the new vaccination date, if applicable.

Statistical analysis

Analysis is by intention-to-treat. Intervention and placebo groups will be compared at baseline for sociodemographic and clinical characteristics, as well as for microbiota composition. Results for continuous variables will be presented as means±SD or as geometric means and 95% CI and for categorical variables, as N (%). For the primary outcome, we will consider antibody level as a dichotomous variable (detectable, undetectable) and use a generalised method of moments logistic regression model with time-dependent covariates for longitudinal binary data. The model will be stratified in subgroup analyses by age and sex at three different timepoints (inclusion; 3 and 6 months post-vaccination). If anti-S1-RBD antibodies as a quantitative variable is available, a GEE regression model with appropriate link function for continuous outcome will be used. Generalised linear models will be used to assess the effect of the group at specific timepoints (3 months and 6 months). The outcomes at the three timepoints are the interaction between group (intervention vs control) and timepoint.[41] Secondary outcomes at inclusion and at 6 months post-vaccination will be analysed on log-transformed original measures for continuous variables; at 6 months, groups will be compared using analyse

of covariance (ANCOVA) with inclusion data covariates. CD4 and CD4/CD8 ratios with log-transformed antibody levels will be estimated using Spearman's correlation and 95% CIs. P values <0.05 will be considered statistically significant. For COVID-19 confirmed cases, a χ^2 test of independence will be used to compare the proportion of symptomatic and asymptomatic participants between the study groups. Cox proportional hazard models will be used to compare the risk of a COVID-19 outcome between the study groups, measured at six different timepoints.

Sample size calculation

We calculated the sample size to discern an effect size of 33% reduction in the number of participants presenting undetectable levels of anti-S1-RBD antibodies at 6 months post-vaccination. With an expected undetectable antibody level in 30% of the placebo group and therefore 20% in the intervention group, an estimated 584 participants would provide 80% power and a two-tailed alpha of 5%. With an estimated 15% attrition rate, the study would therefore require 688 participants.

Patient and public involvement statement

The study will involve the Patient-Partnership Strategic Committee (*Comité stratégique patient-partenaire*, CSPP) of the CR-CHUS, coordinator C. Wilhelmy and the Patient-Partnership Initiative (*Initiative patient-partenaire FMSS-UdeS et Réseau Universitaire Intégré de Sherbrooke*), M. Garriss. In collaboration with the *CIUSSS-Estrie* living lab for geriatric research (*Laboratoire d'innovations par et pour les aînés*, LIPPA), seniors will have the opportunity to join focus groups, collaborate at various stages of the research, and participate in the pilot study. We are also grateful to the National COVID-19 Clinical Trials Network, led by R. Fowler, and the Quebec Research Network on Aging, led by P. Gaudreau, for their support.

Ethics and dissemination

The study was approved by Research Ethics Board of the *Centre Intégré Universitaire de Santé et des Services Sociaux de l'Estrie- Centre Hospitalier Universitaire de Sherbrooke* (*CIUSSS de l'Estrie-CHUS*) and the *CHU de Québec-Université Laval # MP-31-2022-4598*. Written informed consent of all participants will be obtained for participation in the study and publication of results (see online supplemental material). National and international regulations on participant privacy and rights will be followed.

Participants will receive compensation for their participation in the study. One payment at mid-study and another one at the end. Participants who travel to the clinical research centre *CR-CHUS* for venous blood samples or to obtain help from the research team for dried blood spot samples will receive a compensation supplement.

The results of the present study will be published in peer-reviewed journals and presented at international meetings/committees. We will also prepare communications for professionals and the general public.

DISCUSSION

This double-blind randomised controlled trial will test a probiotic supplement as an adjuvant for enhancing COVID-vaccine immune response in the elderly population. Improved immune response and extended spacing of vaccine booster doses in high-risk populations are both major public health issues. If successful, probiotic adjuvants could rapidly be implemented worldwide. They have few side effects, are currently available without prescription and are relatively affordable. Furthermore, if a probiotic supplement can act as a COVID-19 vaccine adjuvant to enhance immunity, this low-cost intervention could lead to important benefits such as hospitalisation reductions, longer time between booster shots and higher immunity against new variants. This intervention would then be clinically, socially and economically beneficial.

Author affiliations
[1]Department of Obstetrics & Gynaecology, Université de Montréal, Montreal, Québec, Canada
[2]CHUM, Montreal, Québec, Canada
[3]Université de Sherbrooke, Sherbrooke, Quebec, Canada
[4]Laval University, Quebec City, Québec, Canada
[5]Direction de la Santé Publique de l'Estrie-CIUSS de l'Estrie-CHUS, Sherbrooke, Quebec, Canada
[6]Social and Preventive Medicine, Université de Montréal Faculté de Médecine, Montreal, Québec, Canada
[7]Centre Hospitalier Universitaire de Sherbrooke, Sherbrooke, Quebec, Canada
[8]Graduate and Post-doctoral studies, Université de Montréal, Montreal, Québec, Canada

Collaborators The study will involve the Patient-Partnership Strategic Committee (*Comité stratégique patient-partenaire, CSPP*) of the CRCHUS, coordinator C. Wilhelmy and the Patient-Partnership Initiative (Initiative patient-partenaire FMSS-UdeS et Réseau Universitaire Intégré de Sherbrooke), M. Garriss. In collaboration with the *CIUSSS-Estrie* living lab for geriatric research (Laboratoire d'innovations par et pour les aînés, LIPPA), seniors will have the opportunity to join focus groups, collaborate at various stages of the research, and participate in the pilot study. We are also grateful to the National COVID-19 Clinical Trials Network, led by R. Fowler, and the Quebec Research Network on Aging, led by P. Gaudreau, for their support.

Contributors Co-appliant to the funding grant application (supervised by J-CP, principal applicant): MP, SR, NC, GB, IL-L, GB, TF, MG, BM, JR, HA-C, AP, J-FB. Drafting of the manuscript: DHB. Critical revision of the protocol and manuscript for important intellectual content: all authors. Substantial contributions to interpretation: all authors. Substantial contributions to statistical analysis plan: BM. J-CP is the guarantor.

Funding This work was supported by a Canadian Institutes of Health Research (CIHR) grant, protocol # EG9-179473.

Competing interests There are no conflicts of interest to declare for JC Pasquier, M Plourde, S Ramanathan, N Chaillet, G Boivin, I Laforest-Lapointe, G Baron, T Fülöp, M Généreux, B Mâsse, J Robitaille, L Valiquette, JF Beaulieu, D. Buch. H Allard-Chamard received some honoraria from Merck for long COVID conferences. A Piché had grants from the Canadian Institutes of Health Research for his work on long COVID prevention, complications, and treatment. He also received honoraria as speaker from the Congrès Québécois en Santé Respiratoire 2023, the Journée de la pharmacologie 23 and at the Fédération des Médecins Spécialistes du Québec.

He also participates in a working group for the Canadian guidelines for Post COVID condition and he is a member of the steering committee of the BQC19 (Biobanque québécoise de la COVID-19).

Patient and public involvement Patients and/or the public were involved in the design, conduct, reporting or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by The study was approved by the Research Ethics Boards of the Sherbrooke Hospital Integrated University Health and Social Services Centre, Estrie (CIUSSS de l'Estrie – CHUS) #MP-31-2022-4598 and the CHU de Québec-Université Laval # MEO-31-2022-6278. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

ORCID iD

Sarah Bilodeau http://orcid.org/0009-0007-6182-5902

REFERENCES

1 Breznik JA, Zhang A, Huynh A, *et al*. Antibody Responses 3-5 Months Post-Vaccination with mRNA-1273 or BNT163b2 in Nursing Home Residents. *J Am Med Dir Assoc* 2021;22:2512–4.
2 Shi C, Wang L, Ye J, *et al*. Predictors of mortality in patients with coronavirus disease 2019: a systematic review and meta-analysis. *BMC Infect Dis* 2021;21:663.
3 Salimi S, Hamlyn JM. COVID-19 and Crosstalk With the Hallmarks of Aging. *J Gerontol A Biol Sci Med Sci* 2020;75:e34–41.
4 Goldberg Y, Mandel M, Bar-On YM, *et al*. Waning Immunity after the BNT162b2 Vaccine in Israel. *N Engl J Med* 2021;385:NEJMoa2114228.
5 Chung JY, Thone MN, Kwon YJ. COVID-19 vaccines: The status and perspectives in delivery points of view. *Adv Drug Deliv Rev* 2021;170:1–25.
6 Flaxman A, Marchevsky NG, Jenkin D, *et al*. Reactogenicity and immunogenicity after a late second dose or a third dose of ChAdOx1 nCoV-19 in the UK: a substudy of two randomised controlled trials (COV001 and COV002). *Lancet* 2021;398:981–90.
7 Painter MM, Mathew D, Goel RR, *et al*. Rapid induction of antigen-specific $CD4^+$ T cells is associated with coordinated humoral and cellular immunity to SARS-CoV-2 mRNA vaccination. *Immunity* 2021;54:2133–42.
8 Cho A, Muecksch F, Schaefer-Babajew D, *et al*. Anti-SARS-CoV-2 receptor-binding domain antibody evolution after mRNA vaccination. *Nature New Biol* 2021;600:517–22.
9 Israel A, Shenhar Y, Green I, *et al*. Large-Scale Study of Antibody Titer Decay following BNT162b2 mRNA Vaccine or SARS-CoV-2 Infection. *Vaccines (Basel)* 2021;10:64.
10 Vicenti I, Basso M, Gatti F, *et al*. Faster decay of neutralizing antibodies in never infected than previously infected healthcare workers three months after the second BNT162b2 mRNA COVID-19 vaccine dose. *Int J Infect Dis* 2021;112:40–4.
11 Xu K, Wang Z, Qin M, *et al*. A systematic review and meta-analysis of the effectiveness and safety of COVID-19 vaccination in older adults. *Front Immunol* 2023;14:1113156.
12 Sadarangani M, Marchant A, Kollmann TR. Immunological mechanisms of vaccine-induced protection against COVID-19 in humans. *Nat Rev Immunol* 2021;21:475–84.
13 Abe KT, Hu Q, Mozafarihashjin M, *et al*. Neutralizing antibody responses to sars-cov-2 variants in vaccinated ontario long-term care home residents and workers. *Infectious Diseases (except HIV/AIDS)* [Preprint] 2021.
14 Sominsky L, Walker DW, Spencer SJ. One size does not fit all - Patterns of vulnerability and resilience in the COVID-19 pandemic and why heterogeneity of disease matters. *Brain Behav Immun* 2020;87:1–3.
15 Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. *Cell Res* 2020;30:492–506.
16 Wilson AS, Koller KR, Ramaboli MC, *et al*. Diet and the Human Gut Microbiome: An International Review. *Dig Dis Sci* 2020;65:723–40.
17 Stavropoulou E, Bezirtzoglou E. Probiotics in Medicine: A Long Debate. *Front Immunol* 2020;11:2192.
18 Din AU, Mazhar M, Waseem M, *et al*. SARS-CoV-2 microbiome dysbiosis linked disorders and possible probiotics role. *Biomed Pharmacother* 2021;133:110947.
19 Haran JP, McCormick BA. Aging, Frailty, and the Microbiome-How Dysbiosis Influences Human Aging and Disease. *Gastroenterology* 2021;160:507–23.
20 Hagan T, Cortese M, Rouphael N, *et al*. Antibiotics-Driven Gut Microbiome Perturbation Alters Immunity to Vaccines in Humans. *Cell* 2019;178:1313–28.
21 de Jong SE, Olin A, Pulendran B. The Impact of the Microbiome on Immunity to Vaccination in Humans. *Cell Host Microbe* 2020;28:169–79.
22 Yeh T-L, Shih P-C, Liu S-J, *et al*. The influence of prebiotic or probiotic supplementation on antibody titers after influenza vaccination: a systematic review and meta-analysis of randomized controlled trials. *Drug Des Devel Ther* 2018;12:217–30.
23 Arioz Tunc H, Childs CE, Swann JR, *et al*. The effect of oral probiotics on response to vaccination in older adults: a systematic review of randomised controlled trials. *Age Ageing* 2024;53:ii70–9.
24 Fonollá J, Gracián C, Maldonado-Lobón JA, *et al*. Effects of Lactobacillus coryniformis K8 CECT5711 on the immune response to influenza vaccination and the assessment of common respiratory symptoms in elderly subjects: a randomized controlled trial. *Eur J Nutr* 2019;58:83–90.
25 Zimmermann P, Curtis N. The influence of probiotics on vaccine responses – A systematic review. *Vaccine (Auckl)* 2018;36:207–13.
26 Fernández-Ferreiro A, Formigo-Couceiro FJ, Veiga-Gutierrez R, *et al*. Effects of *Loigolactobacillus coryniformis* K8 CECT 5711 on the Immune Response of Elderly Subjects to COVID-19 Vaccination: A Randomized Controlled Trial. *Nutrients* 2022;14:228.
27 Clinical Trials-Search results COVID-19+Probiotics, Available: https://clinicaltrials.gov/search?term=probiotic&cond=covid-19%20vaccine
28 Pfeffer RI, Kurosaki TT, Harrah CH, *et al*. Measurement of functional activities in older adults in the community. *J Gerontol* 1982;37:323–9.
29 Zinszer K, McKinnon B, Bourque N, *et al*. Seroprevalence of anti-SARS-CoV-2 antibodies among school and daycare children and personnel: protocol for a cohort study in Montreal, Canada. *BMJ Open* 2021;11:e053245.
30 Limoges M-A, Quenum AJI, Chowdhury MMH, *et al*. SARS-CoV-2 spike antigen-specific B cell and antibody responses in pre-vaccination period COVID-19 convalescent males and females with or without post-covid condition. *Front Immunol* 2023;14:1223936.
31 Limoges M-A, Lortie A, Demontier É, *et al*. SARS-CoV-2 mRNA vaccine-induced immune responses in rheumatoid arthritis. *J Leukoc Biol* 2023;114:358–67.
32 Ahava MJ, Kurkela S, Kuivanen S, *et al*. Detection of SARS-CoV-2 nucleocapsid antigen from serum can aid in timing of COVID-19 infection. *J Virol Methods* 2022;302:114469.
33 Almeida ND, Schiller I, Ke D, *et al*. The effect of dose-interval on antibody response to mRNA COVID-19 vaccines: a prospective cohort study. *Front Immunol* 2024;15:1330549.
34 Keeley TJH, Satram S, Ghafoori P, *et al*. Content validity and psychometric properties of the inFLUenza Patient-Reported Outcome Plus (FLU-PRO Plus©) instrument in patients with COVID-19. *Qual Life Res* 2023;32:1645–57.
35 Shatenstein B, Nadon S, Godin C, *et al*. Development and validation of a food frequency questionnaire. *Can J Diet Pract Res* 2005;66:67–75.
36 Bettinger JA, Sadarangani M, De Serres G, *et al*. The Canadian National Vaccine Safety Network: surveillance of adverse events following immunisation among individuals immunised with the COVID-19 vaccine, a cohort study in Canada. *BMJ Open* 2022;12:e051254.

37 Laboratory biosafety guidance related to coronavirus disease (COVID-19): Interim guidance, 2021. Available: https://www.who.int/publications-detail-redirect/WHO-WPE-GIH-2021.1 [Accessed 27 Sep 2023].
38 CDC Labs. Centers for Disease Control and Prevention. 2020. Available: https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html
39 BQC19. Banque québécoise de la COVID-19, Available: https://bqc19.ca/ [Accessed 27 Sep 2023].
40 Manzanares W, Lemieux M, Langlois PL, *et al*. Probiotic and synbiotic therapy in critical illness: a systematic review and meta-analysis. *Crit Care* 2016;19:262.
41 Lalonde TL, Wilson JR, Yin J. GMM logistic regression models for longitudinal data with time-dependent covariates and extended classifications. *Statist Med* 2014;33:4756–69.